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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The proposed research seeks to design and synthesize large organic molecules possessing precisely defined geometric shapes, a capability which will make possible the design of catalysts and receptors. The approach entails the design of unnatural amino acids which, when incorporated into peptides, will enforce tertiary structure on the overall molecule. Methods are also suggested whereby transiently stable noncovalent complexes of peptides might be permanently stabilized by covalent crosslinking, leading to the concept of "self-assembly" of large, highly ordered organic molecules. The additional possibility of assembling large structures from smaller pieces by incorporation of temporary functionality which will initially spatially orient small pieces prior to covalent assembly is suggested, a concept termed "directed assembly."

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Annual Progress Report on Contract N00014-88-K-0201

Principal Investigator: Paul B. Hopkins

Contractor: University of Washington

Contract Title: Control of Synthetic Peptide Tertiary...

Start Date: 1 February 1988

Current Date: 15 April, 1990

Research Objective:

The design, synthesis, and structural characterization of peptides of 10 to 35 residues with stable secondary and tertiary structure

Progress (Year 2):

As described in the workplan of last year's annual report, we focused our attention this past year on the synthesis and evaluation of a series of peptides which contain two metal-ligating sides chains spaced such that simultaneous chelation of a single metal ion might enhance α-helicity. We chose as our goal the synthesis of eight peptides, $AcAdaAla_mAda(Ala_4GluLys)_3NH_2$, in which Ada is an L-$\alpha$-amino acid residue with the aminodiacetic acid side chain, $(CH_2)_nN(CH_2CO_2H)_2$, and values of m and n are all combinations of m=2,3 and n=1-4. Half of these syntheses are now complete. The syntheses require, at the outset, the availability of the four L-α-amino acids represented by Ada. These compounds were synthesized as the N^{α}-Boc-Ada(O^{ε}Bzl)$_2$-protected derivatives and were incorporated into peptides by solid phase methods in either a fully stepwise synthesis, or, in a few cases, by a block approach. The resulting peptides were purified by reverse phase HPLC. FABS MS analysis of the purified peptides afforded the expected parent ion; for one peptide (prepared without terminal N-acetylation) sequence analysis confirmed the expected structure.

Circular dichroism analysis of these synthetic peptides is currently in progress. The limited available information suggests that the tether length (m) and spacing between ligating residues (n) are critical. As expected, long tethers apparently decouple the ligation of the metal from significant effect on the conformation of the attached peptide. The impact of the shortest tethers is at present unknown. Limited data also suggest that the spacing between ligating residues is critical, with n=2 being apparently too short to allow helix formation and actually destabilizing the α-helical form. The

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equilibrium ratio of helix to random coil was increased three fold by addition of Cu^{2+} to one of these peptides. Whether this is the greatest enhancement for the familh of eight peptides will be known only on completion of the syntheses and further circular dichroism analysis.

Workplan (Year 3):

Having (at last!) prepared a family of peptides which exihibit metal ion-dependent conformational properties, the focus of the project will now shift to evaluation of the solution conformational properties of these peptides. We will determine the CD spectra from 180 to 260 nm in the presence of a wide variety of metal ions (Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}). Theory predicts that a maximum shift of about 10^5 of the equilibrium constant in favor of α-helix could be observed; of the three peptides we have thus far evaluated we have seen at most a factor of 3. Based upon those values of m and n (see above) which are found to provide the most enhanced helicity, we may elect to synthesize one or two more peptides in which each peptides bears two different values of n. The goal of this would be to optimize the stabilization of the helical form. We might, for example, prepare peptides in which the first ligand is held with an ethylene and the second with a propylene tether and vice versa. For those combinations of peptide/metal which are found to be interesting by CD, we will study the impact of pH on the conformational equilibria. The state of aggregation of these peptides in their helical form (expected to be monomers) will be verified by gel filtration experiments. Nuclear magnetic resonance studies will be undertaken to verify the helical structure evident by CD. Finally, computer molecular modeling studies, using molecular mechanics techniques, will be undertaken to attempt to rationalize the observed trends in helix stabilizaiton as a function of tether length and inter-residue spacing.

Inventions:

None

Publications:

Hopkins, Paul B. Hopkins, Ruan, F.; Chen, Y.; Sasaki, T.; and Itoh, K., "Metal Ion-Induced α-Helicity in Synthetic Peptides Containing Unnatural, Metal-Ligating Residues," Abstract (submitted) 200th National Meeting of the American Chemical Society, Washington D.C., August 26-31, 1990.

As soon as circular dichroism studies are complete on the eight peptides described above, we plan to submit one or more manuscripts to the *Journal of the American Chemical Society.*

Training Activities:

Mr. Fugiang Ruan and Dr. Yanqiu Chen. both native born citizens of the Peoples Republic of China are currently working on this project. Mr. Ruan's major focus is peptide synthesis; Dr. Chen is evaluating the solution conformational properties of the synthetic peptides. Professor Tomikazu Sasaki, a new faculty member with considerable experience in the field of peptide design and synthesis, is serving as an informal consultant on the project.

Awards/Fellowships:

The PI is an Alfred P. Sloan Fellow (1988-1992) and is recipient of an NIH Research Career Development Award. Fuqiang Ruan is a PRC Scholar.